H_2O_2-Induced Stress Responses of *Shewanella oneidensis* MR-1

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ABSTRACT

The availability of whole genome microarray for *Shewanella oneidensis* makes it possible to investigate in detail the gene expression on a global scale, allowing genome-wide understanding of the regulatory mechanisms involved in stress responses. In this report we applied the full genome cDNA microarray to study the changes in *Shewanella oneidensis* transcriptome in response to hydrogen peroxide induced oxidative stress. H_2O_2 concentrations and time course were designed to monitor the adaptive and dynamic nature of gene regulation. Data analysis revealed that many genes showed dose-dependent expression pattern and were differentially regulated at different time points. Among the rapidly up-regulated genes were those involved in cellular detoxification processes, such as *ahp*C, *ahp*F, *kat*B, and DNA binding protein *dps*, energy metabolism, as well as genes involved in iron homeostasis and sulfur-limitation response, whose functions in oxidative stress are yet to be clarified. The late transcriptional changes included the induction of SOS repair genes, prophage genes and heat shock and chaperonin proteins. Comparison of the low dose and high dose treatment data suggested that stronger H_2O_2 stimulus induced both H_2O_2 specific response and general stress response, which was characterized by the down regulation of the translational and transcriptional machinery genes and the re-programming of the aerobic and anaerobic metabolism pathways. To better understand the regulatory mechanism that controls the transcriptional response, we used computational methods and identified the dominant regulatory elements for each experimental condition; in combination with operon prediction and comparative genomics analysis we were able to predict the major regulatory mechanisms that underlie the complex expression changes. In an effort to further examine our regulatory model we employed mutagenesis, microarray, and computational modeling to confirm the major regulatory role of *fur* gene in modulating H_2O_2 stress response and identified a dual function *oxy*R homologue in *Shewanella onedensis* genome.

EXPERIMENTAL DESIGN

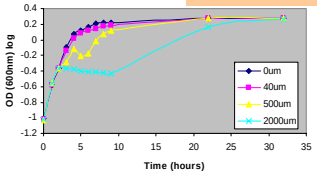

Fig. 1 Cell growth under oxidative stress. Various concentrations of H_2O_2 was added to log phase culture in LB medium. Growth kinetics was measured through optical density at 600 nm wavelength. Results represent the trend of multiple individual experiments. 40 and 50 μM of H_2O_2 were selected for later experiments.

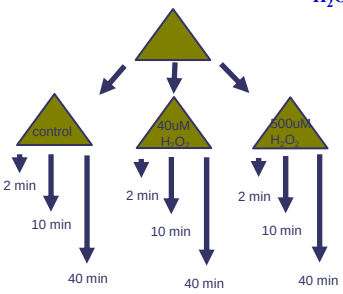

Fig. 2 Experimental design. Early to mid-log phase aerobic cells were aliquot into three portions and treated with mock,40uM H_2O_2,and 500uM H_2O_2 respectively. Cells were harvested in parallel to avoid handling differences. RNAs extracted from these samples were used in subsequent microarray hybridizations and RT-PCRs.

ACKNOWLEDGEMENTS

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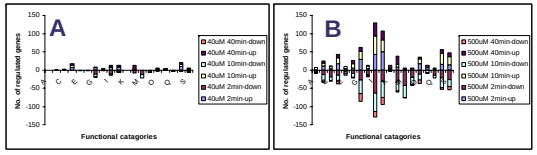
RESULTS

Overview of the transcriptome changes Total number of differentially expressed genes under various conditions

		No. of genes	
Teatment time	Regulation	40uM H2O2	500uM H2O2
2min	Up	47	236
	Down	69	364
10min	Up	23	249
	Down	22	356
40min	Up	33	159
	Down	10	114



TIGR functional catagories

A	Amino acid biosynthesis	K	Other categories
B	Biosynthesis of cofactors, prosthetic groups, and carriers	L	Protein fate
C	Cell envelope	M	Protein synthesis
D	Cellular processes	N	Purines, pyrimidines, nucleosides, and nucleotides
E	Central intermediary metabolism	O	Regulatory functions
F	DNA metabolism	P	Signal transduction
G	Energy metabolism	Q	Transcription
H	Fatty acid and phospholipid metabolism	R	Transport and binding proteins
I	Hypothetical proteins (conserved)	S	Unknown function
J	Hypothetical proteins		

Fig. 3 Distribution of differentially expressed genes in TIGR functional categories. Differentially regulated genes categorized by functional classification. (A) 40uM H_2O_2, (B) 500uM H_2O_2.

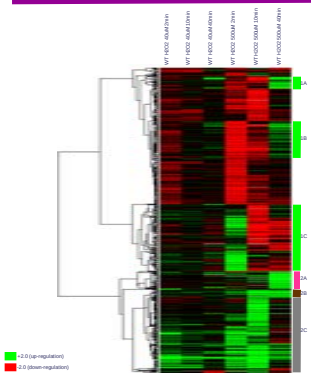


Fig. 4 Hierarchical cluster analysis of microarray data. Clusters 1A and 1B mainly consist of heat shock and chaperonin proteins. 1C contains many energy metabolism genes. 2A represents prophage and heat shock proteins. 2B contains SOS system genes. 2C represents most of the cellular process genes such as *dps*, *ahp*C,F, and *kat*B, as well as iron transport and metabolism genes and sulfur metabolism genes.

Regulatory motifs	Representative genes	Possible TF
	SO3343, SO3030, SO4516, SO2039, SO1482	Fur
	SO1070, SO1158, SO3371, SO1917, SO0958	OxyR
	SO3462, SO4603, SO2604, SO3430, SO0726	LexA
	SO3599, SO3738, SO0694, SO4480	CysB??
	SO0703, SO1126, SO1796, SO2016, SO4157	RpoH

Fig. 5 Computational prediction of the major regulatory elements. The intergenic sequences of the differentially expressed genes are subjected to transcriptional binding site prediction using MEME and BioProspector. The sequence logos of the binding sites were generated using WebLogo.

Fig. 5 Low aerobic tolerance of *fur* and SO1328 deletion mutants. Log phase cells were serially diluted and spotted onto LB plates with or without catalase. Δfur and ΔSO1328 cells show severe aero-sensitivity and the growth deficiency can be rescued by catalase.

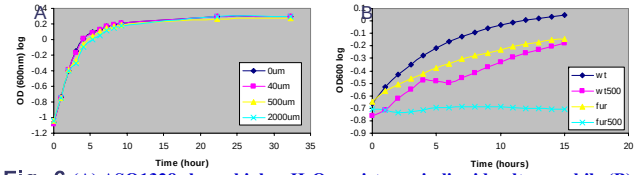
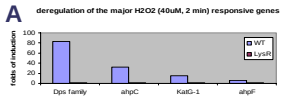
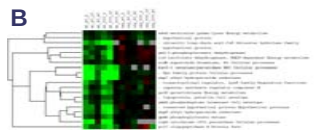


Fig. 6 (A) ΔSO1328 shows higher H_2O_2 resistance in liquid culture, while (B) Δfur remains sensitive.

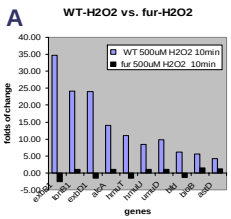




C

Gene	Gene name	Gene Product	Fold change (oxyR/WT)
SO1158	-	Dps family protein	26.2
SO1070	katB	catalase	4.1

Fig. 7 SO1328 acts as a dual function regulator. (A) deregulation of the major H_2O_2 responsive genes in ΔSO1328. (B) cluster analysis of SO1328 dependent H_2O_2 responsive genes. (C) increased expression of dps and katB in untreated ΔSO1328, suggesting that SO1328 functions as a repressor under steady state growth.



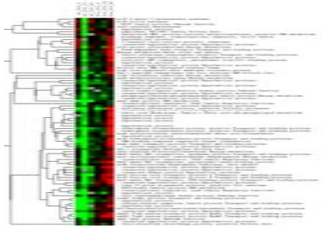


Fig. 8 (A) deregulation of the major H_2O_2 responsive genes in Δfur. (B) cluster analysis of SO1328 dependent H_2O_2 responsive genes.

CONCLUSIONS

1. *Shewanella oneidensis* MR-1 possesses a complex regulatory system to control gene expression under oxidative stress. The major transcriptional responses include the rapid induction of genes involved in antioxidant defense, TonB iron transport, sulfur metabolism, and energy metabolism, and late up-regulation of DNA and protein protection and repair systems.
2. Fur and SO1328 (oxyR) may play essential roles in the regulation of *S. oneidensis* MR-1 responses to H2O2. A proposed model for both TF work is as follows: